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A population genomics insight by 2b-RAD reveals populations' uniqueness along the Italian coastline in *Leptopsammia pruvoti* (Scleractinia, Dendrophylliidae)

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Abstract

Aim: Marine bioconstructions such as coralligenous formations are hotspot of biodiversity and play a relevant ecological role in the preservation of biodiversity by providing carbon regulation, protection and nursery areas for several marine species. For this reason, the European Union Habitat Directive included them among priority habitats to be preserved. Although their ecological role is well established, connectivity patterns are still poorly investigated, representing a limit in conservation planning. The present study pioneers a novel approach for the analysis of connectivity in marine bioconstructor species, which often lack suitable genetic markers, by taking advantage of next-generation sequencing techniques. We assess the geographical patterns of

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genomic variation of the sunset cup coral *Leptopsammia pruvoti* Lacaze-Duthiers, 1897, an ahermatypic, non-zooxanthellate and solitary scleractinian coral species common in coralligenous habitats and distributed across the Mediterranean Sea.

Location: The Italian coastline (Western and Central Mediterranean).

Methods: We applied the restriction site-associated 2b-RAD approach to genotype over 1,000 high-quality and filtered single nucleotide polymorphisms in 10 population samples.

Results: The results revealed the existence of a strongly supported genetic structure, with highly significant pairwise F_{ST} values between all the population samples, including those collected about 5 km apart from each other. Moreover, genomic data indicate that the strongest barriers to gene flow are between the western (Ligurian–Tyrrhenian Sea) and the eastern side (Adriatic Sea) of the Italian peninsula.

Main conclusions: The strong differentiation found in *L. pruvoti* is similar to that found in other species of marine bioconstructors investigated in this area, but it strongly contrasts with the small differences found in many fish and invertebrates at the same geographical scale. All in one, our results highlight the importance of assessing connectivity in species belonging to coralligenous habitats as, due to their limited dispersal ability, they might require specific spatial conservation measures.

KEYWORDS

bioconstructors, Italian coastline, marine protected areas, population genomics, single nucleotide polymorphisms

1 | INTRODUCTION

Marine conservation strategies are nowadays widely recognized to require a holistic approach that implies the shifting from the conservation of single species to that of entire ecosystems and their functions (Long, Charles, & Stephenson, 2015). Moreover, the application of sound conservation measures should be targeted to management units, identified through the analyses of ecological coherence and connectivity (Almany et al., 2009; Boero et al., 2016). For the European marine realm, this ambitious goal is well interpreted by the Marine Strategy Framework Directive (MSFD; 2008/56/EC; European Commission, 2008), which emphasizes the need of maintaining biological diversity ensuring a Good Environmental Status (GES). Similarly, also the Habitat Directive (HD; 92/43/EEC, European Commission, 1992) aims at maintaining or restoring a favourable conservation status focusing on selected marine habitats (among which coralligenous formations) and preserving their species composition, structures and functions in the long term.

The establishment of opportunely selected marine protected areas (MPAs) represents one of the most effective approaches for marine biodiversity conservation (Gaines, White, Carr, & Palumbi, 2010; Micheli & Halpern, 2005; Pascual, Rives, Schunter, & Macpherson, 2017). MPAs mainly aim at the local reduction of threats by human impacts, thus promoting biological recovery over time and, consequently, protecting and increasing biodiversity (Benedetti-Cecchi et al., 2003; Katsanevakis et al., 2011; Lubchenco, Palumbi, Gaines, & Andelman, 2003). Important benefits due to biodiversity spillover may also interest neighbour non-protected sites, favouring an overall recovery of marine habitat status. Moreover, passing from a single-MPA perspective to effective MPA network is considered a present challenge in marine conservation. Thus, the establishment of interconnected MPAs improves and extends the positive effects of assuring the persistence of a good state for biodiversity (Boero et al., 2016; Gaines et al., 2010; Palumbi, 2004), providing the important spatial links needed to maintain large-scale ecosystem processes. Accordingly, the Aichi Target 11 of the Convention on Biological Diversity aims at increasing the proportion of protected areas in the world to establish "a well-connected system of protected areas" by 2020 (Brooks et al., 2006). Ensuring connectivity is also crucial to improve resilience following environmental perturbations, failures in management or other hazards, and thus helping to ensure better long-term sustainability of species (Cowen & Sponaugle, 2009).

The maintenance of biodiversity at the local scale is critically dependent on the persistence of populations, influenced by self-recruitment and propagule supply that are, to a wide extent, species-specific processes. Connectivity itself, which depends on ocean circulation, life history traits, local population dynamics and spatial distribution of populations (Jonsson & Watson, 2016), is largely determined by specific population processes, and it is often inferred, for single species, at the metapopulation level (Dalongeville, Andrello, Mouillot, Albouy, & Manel, 2016).

Ideally, the design (location and size) of a robust MPA network should be supported by connectivity estimates for all the species of a given community to identify a consensus network which ensures enough connectivity to multiple species with different life history traits (Costantini, Ferrario, & Abbiati, 2018; Dalongeville et al., 2016; Jonsson & Watson, 2016; Pascual et al., 2017; Shanks, Grantham, & Carr, 2003). Recently, Melià et al. (2016) showed a novel methodological framework to synthesize species-specific results into a set of community connectivity metrics and showed that spatiotemporal variation in magnitude and direction of the connections and interspecific differences in dispersing traits are key factors structuring community connectivity. However, these attempts are still confined to few examples given the costs, technical limitations of commonly used methods for connectivity inference, and critical gaps in knowledge.

Given the above reasons, connectivity estimates should at least focus on species with critical ecological role and, among these, on habitat formers, which per se structure the local communities (Boero et al., 2016). Among marine habitats, in fact, benthic habitats (e.g., seagrass meadows, coralligenous reefs and marine caves) were selected as priority for biodiversity protection and conservation by the HD (Giakoumi et al., 2013).

Coralligenous is among the habitats with the highest species richness in the Mediterranean Sea (Calcinai et al., 2015). It includes habitat-forming species that, growing on pre-existent secondary substrates, build and constantly modify the bottom architecture (Ballesteros, 2006). These continuously evolving surfaces are suitable for the settlement of other bioconstructors and/or for the establishment of nursery grounds of other marine species. This makes bioconstructors key components for the analyses of biodiversity and for studies on connectivity (Ingrosso et al., 2018). Several approaches can be used to infer connectivity in the marine system, and among them, estimation of genetic differentiation is considered one of the most profitable strategies. However, genetic investigation especially on species that contribute to bioconstructions is currently limited by the lack of genetic information for these organisms.

Several recent approaches, based on next-generation sequencing (NGS), offer the possibility to collect an impressive amount of genetic data without the need of a priori information on the species analysed (Hohenlohe, Catchen, & Cresko, 2012). Among these, restriction site-associated DNA sequencing (RAD-seq) methods (Baird et al., 2008), through the reduction of genomic information, allow the simultaneous characterization of a high number of non-randomly selected single nucleotide polymorphisms (SNPs) in hundreds of samples.

In this paper, the 2b-RAD protocol (Wang, Meyer, McKay, & Matz, 2012), a member of the RAD-seq family, was optimized for the first time in one bioconstructor species of the Mediterranean basin, the sunset cup coral *Leptopsammia pruvoti* Lacaze-Duthiers, 1897. The 2b-RAD method allowed investigating the geographical pattern of genetic differentiation between 10 population samples collected over about 2,500 km along the Italian coastline (Western and Central Mediterranean Sea). Besides representing about 1/5 of the whole Mediterranean coast, the Italian coastline is in a key position, including the Western and Central Mediterranean basin (FAO areas 37.1 and 37.2).

The scleractinian *L. pruvoti* is an azooxanthellate solitary coral, mainly living in shaded and enclosed rocky habitats, under overhangs, at depth ranging from few metres up to 150 m (Zibrowius, 1980). In the Mediterranean Sea, *L. pruvoti* is the unique exponent of its genus and can frequently reach a density over than 5,000 individuals/m^2, but it was observed to exceed the 17,000 individuals/m^2 (Goffredo, Radetić, Airi, & Zaccanti, 2005). The species is also present in north-eastern Atlantic coasts from Portugal to southern England and Ireland. *Leptopsammia pruvoti* is a gonochoric and brooding coral that reproduces annually. After fertilization, embryos are incubated until maturity in the coelenteric cavity of the female. With planulation, small and completely differentiated larvae (ranging from 695 to 1,595 μm (Goffredo et al., 2005) and characterized by a ciliary movement) are released. No data from field experiments to estimate the larval dispersal are available. However, larvae generated in laboratory have shown that they are ready to settle when released and can swim by ciliary movement for 1–20 days (Goffredo et al., 2005). No evidences for asexual reproduction have been reported (Goffredo, Di Ceglie, & Zaccanti, 2009). The scatter distribution of this species along the Italian coasts might be due not only to a limited dispersal ability of larvae but also to the fragmented distribution of suitable habitats for settlement. Information about the genetic structure of *L. pruvoti* in the Mediterranean Sea is very scarce with the only exception of an allozyme analysis detecting no evidence of a correlation between genetic differentiation and geographical distances (Goffredo et al., 2009).

The present study, besides representing the first large-scale population genomic investigation for *L. pruvoti*, aims at the following three objectives: (a) assessing the geographical patterns of genomic variation within and among populations of *L. pruvoti*; (b) estimating self-recruitment and direct connections among sampling sites using assignment tests; and (c) comparing the obtained results with those available from other bioconstructor species, to improve the management of existing MPAs and to support the planning of sites needing protection.

2 | METHODS

2.1 | Sample collection and genomic DNA extraction

A total of 295 individuals of *L. pruvoti* were collected from 10 sampling sites in the Mediterranean Sea (three in the Adriatic Sea, five in the Tyrrhenian Sea and two in the Ligurian Sea; Table 1 and Figure 1). At each site, the polyps were sampled at depth between 10 and 30 m by scuba-diving. Whenever possible, individuals were collected at a minimum distance of some metres to each other, in order to reduce the risk of sampling of highly related specimens due to self-recruitment. Unfortunately, the precise collection depth of each animal was not recorded, preventing the possibility to identify possible

TABLE 1 Sampling information of *Leptopsammia pruvoti* populations collected between 2014 and 2015 at 10 locations along the coasts of the Italian peninsula in the Mediterranean Sea. For each sampled population, the following data are reported: sea, location, acronym, geographical coordinates, number of individuals sampled (*N*) and successfully analysed (Na) and sampling depth. Localities identified as marine protected areas (MPAs) are marked with asterisks. Geographical coordinates are in WGS84

			Geographical coordinates				Depth (m)	
Sea	Location	Acronym	Lat. N	Lon. E	*N*	Na	Min	Max
Adriatic	Tegnùe_P208b*	TGN	45°11′40″	12°25′29″	35	28	19	21
Adriatic	Tremiti Islands (Capraia, Secca della Vedova)*	TR	42°07′59″	15°30′24″	42	22	15	25
Adriatic	Otranto	OT	40°06′21″	18°33′50″	26	22	15	20
Tyrrhenian	Trapani (Riserva dello Zingaro)*	TP	38°04′42″	12°55′32″	36	34	15	20
Tyrrhenian	Capo Spartivento	CSP	38°52′38″	08°50′18″	35	21	17	20
Tyrrhenian	Tavolara Island*	TAV	40°55′24″	09°42′06″	30	19	10	30
Tyrrhenian	Giannutri South (I Grottoni)*	GNS	42°14′20″	11°06′13″	21	13	10	30
Tyrrhenian	Giannutri North (Punta Secca)*	GNN	42°15′49″	11°06′35″	18	12	10	30
Ligurian	Portofino (Punta del Faro)*	PTF	44°17′54″	09°13′06″	32	26	15	25
Ligurian	Gallinara (Punta Falconara)	GA	44°01′21″	08°13′35″	20	15	10	25
Total					295	212		

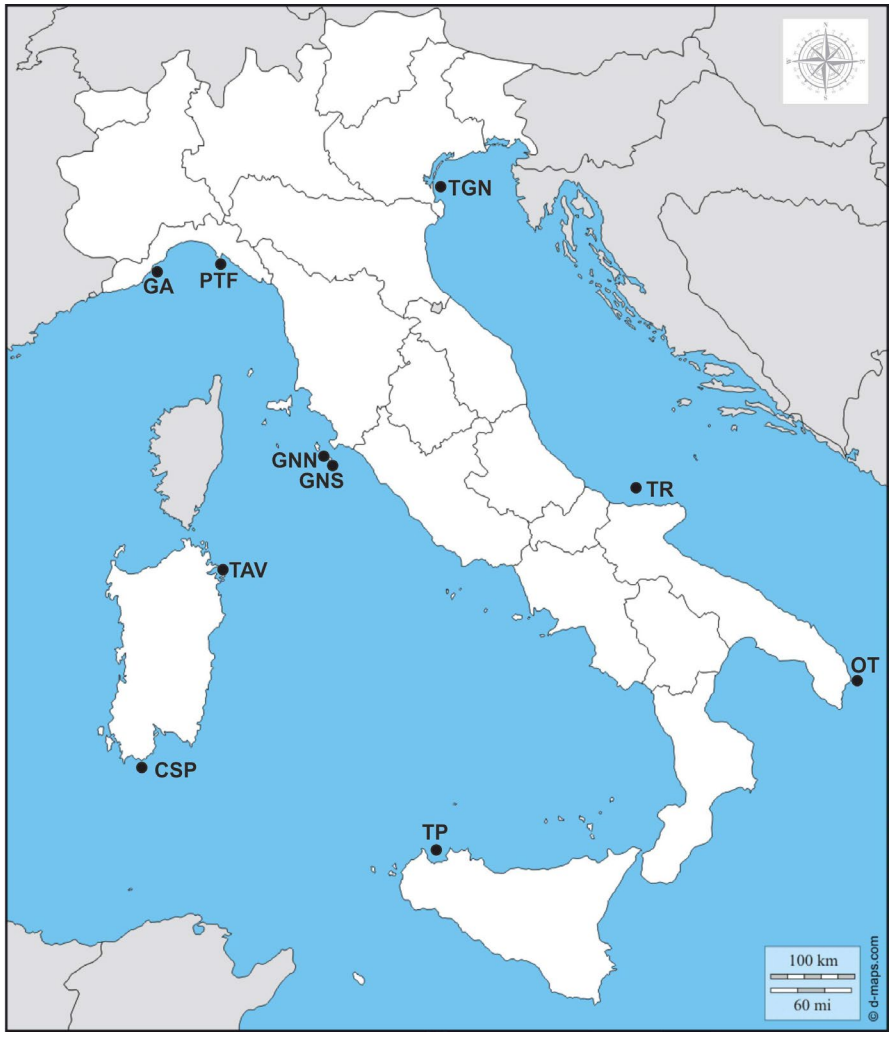

FIGURE 1 Sampling sites in the Mediterranean Sea. See Table 1 for the correspondence of location acronyms

vertical genetic differentiation due to adaptation to different depths (Pratlong et al., 2018). Once collected, all samples were preserved in individual vials with 99% absolute ethanol at −20°C.

Total RNA-free genomic DNA (gDNA) was extracted from all the specimens using the *EuroGOLD Tissue DNA Mini Kit* (Euroclone), following the manufacturer's protocol, with a final elution in 100 µl of water. For all the samples, concentration and quality of the extracted DNA were estimated by NanoDrop UV-Vis spectrophotometer 2000c (Thermo Scientific), while DNA integrity was checked on 1% agarose gel in TAE 1× stained with GelRed (BIOTIUM, GelRed™ Nucleic Acid Stain, 10,000× in Water). All the DNA extracts with concentrations below 40 ng/µl were further concentrated by isopropanol precipitation. To this end, 1 volume (V) of isopropanol and 1/10 V of sodium acetate were added to each extract, followed by incubation at −20°C for 2 hrs and centrifugation at maximum speed for 30 min. The DNA pellet was rinsed with 500 µl of 70% ethanol and, after a centrifugation of 5 min, resuspended in 15 µl of sterile water and quantified by NanoDrop.

2.2 | 2b-RAD-library preparation, sequencing and post-processing of raw reads

Genome-wide single nucleotide polymorphisms were obtained and genotyped using 2b-RAD (Wang et al., 2012). In the present work, the 2b-RAD protocol applied for populations of *Paracentrotus lividus* (Paterno et al., 2017) has been optimized for bioconstructor species with skeleton (see Supporting Information Appendix S1).

A total of 212 gDNAs were successfully processed and merged in two pools for sequencing. Five individuals were processed in duplicate (technical replicates) and included in the sequenced pools for a total of 217 individual libraries. The first pool, composed of 50 individual libraries, was sequenced twice, while for the second one, obtained merging 167 PCR products from different individuals, the sequencing was repeated three times. The recalibration of the relative amount of different 170-bp target bands for each run was performed as described in Paterno et al. (2017).

The pools were sequenced on an Illumina HiSeq platform with a single-end 50-bp read module (SR50 High Output mode) by Genomix4Life S.r.l. (Baronissi, Salerno, Italy), which also performed demultiplexing by individual barcode and quality filtering. Demultiplexed reads were checked for quality by FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Then, the raw reads obtained for each individual library were processed using a custom-made Python script that allowed filtering for the presence of the specific *Csp*CI restriction site and adaptors' trimming. By cutting 10 bases before and 10 bases after the recognition site, fragments of uniform length (32 bp) were generated excluding all positions involved in the adapters' ligation reaction (see Supporting Information Appendix S1).

2.3 | De novo SNP discovery, genotyping and filtering of loci

The STACKS software version 1.35 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013) was used to identify SNPs and to reconstruct individual genotypes, by first identifying loci and their alleles in each individual, and then creating a *Catalog* of all the possible loci from the entire set of individuals. Reads were processed with the *denovo_map.pl* pipeline, without the use of a reference genome. Four out of five technical replicates were used to optimize the assembly parameters (Supporting Information Appendix S1), finally set as follows: minimum stack depth (*m*) of 3, number of mismatches allowed between stacks of the same individual (*M*) equal to 2, and number of mismatches allowed between loci of different individuals to be merged in the final Catalog (*n*) equal to 3. The SNPs were called using the maximum-likelihood statistical model implemented in STACKS (Catchen et al., 2013; Hohenlohe et al., 2010, 2012) considering a *bounded SNP calling model* with an error rate to call SNP between 0 and 0.1 and the significance level required to call a heterozygote (α value) of 0.1.

The STACKS package *Populations*, with the *write_single_snp* option, was used to export genotypes in genepop format for further analysis. Only polymorphic loci containing from 1 to 3 SNPs with a maximum of six alleles and genotyped in at least the 90% of the individuals were exported, as they result the subset that minimize the locus error rate between replicates. When a locus had two or three SNPs, only the first one was retained.

The exported panel of loci was further filtered in order to remove putative sequencing errors, paralogue sequences and uninformative SNPs. First, all loci with a minor allele frequency (MAF) ≤0.01 were discarded as putative sequence errors and/or no informative polymorphisms (Roesti, Hendry, Salzburger, & Berner, 2012). Second, an exact test for Hardy–Weinberg equilibrium (HWE) was conducted in GENEPOP version 4.6 (Rousset, 2008) for each population. Loci showing a significant departure from equilibrium within populations, after Fisher's combined probability test, were removed from the dataset. They might be duplicated loci that could affect the estimate of observed heterozygosity and could bias tests for selection (Roesti et al., 2012).

The final dataset, used for downstream analysis, included 1,386 filtered loci.

2.4 | Statistical analysis of population genomic data

2.4.1 | Genetic diversity

The presence of identical multilocus genotypes (MLGs) was excluded using GENALEX 6 (Peakall & Smouse, 2006). In order to evaluate the possible occurrence of clonal reproduction, the distribution of pairwise genetic distances among individuals was plotted and compared with values obtained from replicates (Supporting Information Appendix S2). Genetic variability within each population and for the total sample was quantified as rarefacted allelic richness (A_R, number of alleles) and private allelic richness (pA_R) using HP-RARE 1.1 (Kalinowski, 2005). The smallest size of the Giannutri South (GNS) population (N = 12) was used to standardize the number of alleles. Unbiased expected (H_E) and observed (H_O) heterozygosity and inbreeding coefficients (F_{IS}) were calculated using GENETIX 4.05.2 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2000). Departure from HWE within

populations for each locus and overall loci was estimated using the Markov chain method (dememorizations = 10,000; batches = 500; iterations = 10,000) in GENEPOP. The significant threshold was set to 0.05, and adjusted by the Benjamini and Hochberg (1995) correction for multiple tests when needed.

2.4.2 | Inference of population structure

Prior to population structure analyses, the statistical power to detect genetic differentiation and the neutrality of markers were assessed. A high power to detect F_{ST} values as low as 0.001 and 5 outliers were detected (see Supporting Information Appendix S3). Analyses were performed considering first the original dataset with 1,386 loci and then a second dataset considering only the 1,381 neutral markers detected. However, all data reported in the Results 2.4 section are referred to the original dataset as they do not significantly differ from results obtained considering only neutral markers.

ARLEQUIN 3.5 (Excoffier, Hofer, & Foll, 2009; Excoffier & Lischer, 2010) was used to estimate pairwise F_{ST} statistics and to perform non-hierarchical and hierarchical analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992). For the above analyses, the locus-by-locus option was used, due to the presence of missing data in the filtered datasets. Significance was assessed by 10,000 permutation tests and *p*-values corrected for multiple tests as above reported for the HWE. The values of genetic differentiation (F_{ST}), obtained with and without loci under selection, were compared by a Mantel permutation test using GENALEX.

The Discriminant Analysis of Principal Components (DAPC; Jombart, Devillard, & Balloux, 2010) implemented in the R package "adegenet" (Jombart, 2008) was used to represent genetic differentiation among estimated clusters (*K*) composed of genetically related individuals. The optimal number of *K*, maximizing the variation among groups, was estimated with the *find.clusters* function. The optimal number of principal components (PCs) that should be retained to perform a DAPC and avoid production of results based on unstable parameters was estimated using the *optim.a.score* function. The analysis was also repeated with prior information about populations' subdivision to provide a visual assessment of between-populations differentiation.

2.4.3 | Comparison between genetic and geographical information

To assess whether there was a pattern of isolation by distance (IBD), the correlation between genetic divergence among populations (pairwise F_{ST} estimates) and pairwise geographical (waterway) distances was tested using a Mantel permutation test implemented in GENALEX with 9,999 permutations. The IBD pattern was first assessed on the whole dataset and repeated excluding the most genetically distant site (Tegnùe sample) to avoid biased measures due to its strong divergence from all other populations. Pairwise shoreline distances between populations were estimated in km using Google Earth (https://www.google.com/intl/it/earth/).

Moreover, the presence of spatial genetic discontinuities was also investigated through a Bayesian assignment method implemented in the R package "GENELAND" v. 4.0.0 (Guillot, Estoup, Mortier, & Cosson, 2005; Guillot, Santos, & Estoup, 2008). The software, considering geographical information as a prior, produces a map in which the assessed most likely number of homogenous genetic clusters (*K*) among sampled individuals is represented. A total of 11 *K* values (ranging from 1 to 11) were tested. For each *K*, five independent runs were performed using the uncorrelated allele frequency model, 212 maximum rate of Poisson process, 636 maximum number of nuclei, 110,000 MCMC repetitions with burn-in period of 100 and a thinning value of 100. The run with the highest mean logarithm of posterior probability was further processed on a landscape of 100 × 100 cells and with a burn-in of 100 iterations.

2.4.4 | Assignment analysis

An assignment test was performed to directly investigate the origin of each individual. The analysis was carried out through the Bayesian exclusion method of Rannala and Mountain (1997) implemented in GeneClass2 (Piry et al., 2004) on a subsample of the original dataset including 422 loci shared by all the 212 individuals, as suggested by the authors. For all the individuals in which the probability of exclusion was under 95%, the hypothesis that they were correctly assigned to their original population has not been rejected. The individuals excluded from their own sampling location were assigned to another sampled population only when the probability of assignment to the new population was above 10% (Underwood, Smith, Van Oppen, & Gilmour, 2007). Otherwise, individuals not assigned to any sampled populations were considered as originated from an unsampled source population (unknown).

3 | RESULTS

3.1 | Sequencing results and filtering

After demultiplexing, a total of 569,408,465 filtered quality raw reads were obtained from Illumina sequencing of the two pools (*N* = 217; five technical replicates were included) with an average of 2,624,002 sequences *per* individuals. A mean of 83.1% reads (2,164,011) *per* individual presented the *Csp*CI restriction site and passed the trimming phase. Thus, a total of 469,590,522 trimmed reads 32 bp long were retained and processed with the *denovo_map.pl* Stacks pipeline to generate the *Catalog* of all the possible loci.

Among 217 individuals, a total of 352,160 2b-RAD tags were identified, 87,838 of which (20%) were polymorphic. A mean of 20,497 tags *per* individual matching the Catalog were obtained (ranging from 10,910 to 54,673), leading to a mean coverage *per* locus of 105 reads. Polymorphisms were detected in a mean of 3,297 loci (16%) *per* individual (ranging from 1,635 to 9,860).

A total of 3,822 loci present in more than 90% of individuals were retained in the first filtering step. Among these, 1,890 loci showed a MAF ≥ 0.01 and were used for the HWE test. Finally, 1,386 loci

(RAD loci with one SNP per locus) were retained for further analysis (except assignment tests which require no missing data; see Methods). At these loci, replicates differed by 3% as a mean number of allele differences between replicates (replicates-threshold) out of the 1,386 analysed loci (2,772 alleles). This was principally caused by the lack of one allele in heterozygote genotypes or the complete absence of a locus in one of the replicates.

3.2 | Population genomic analysis

3.2.1 | Genetic diversity

No identical multilocus genotypes were detected with a minimum of seven loci differing between two individuals (28 when excluding the highly homogenous TGN population; see below). Different MLGs are expected because of sequencing errors or mutation events even in case of clonality whose detection is not straightforward using NGS. Plotting the distribution of pairwise genetic differences among samples (Supporting Information Appendix S2), comparisons within TGN, GA and partially CSP lay out from the general pattern showing similarity values comparable with the ones obtained between replicates. Even if asexual reproduction has never been reported in this species (Goffredo et al., 2009), our result raises the hypothesis that a certain degree of clonal reproduction, observed in other solitary scleractinian corals (Tokuda, Haraguchi, & Ezaki, 2017 and references therein), might occur also in *L. pruvoti* and would deserve a dedicated investigation. In order to exclude a possible effect of this phenomenon on the assessment of population structure and connectivity, analyses were also performed by excluding the putatively clonal genotypes and results compared with the ones obtained from the complete dataset (Supporting Information Appendix S2). Considering that no significant differences were observed, here we report genetic analyses performed using all the 212 sequenced individuals (considering replicates only once).

Summary statistics of genetic variability are reported in Table 2. The percentage of polymorphic SNPs ranged from 6.2 to 47.3, which indicates that the majority of the identified SNPs were fixed within population samples. Accordingly, allelic richness (A_R) and expected heterozygosity (H_E) were low. The CPS population sample showed the highest allelic richness (A_R = 1.34) and gene diversity (H_E = 0.1205). Conversely, the TGN population sample is ranked lower, with values 1.04 and 0.0150 respectively for A_R and H_E and resulted, in general, the sample with the lowest genetic variation (Table 2). Private allelic richness (pA_R) was comparable across samples (range 0.01–0.05) except for the TP site (pA_R = 0.1) where it pointed to the highest genetic distinctiveness (Table 2).

Inbreeding coefficients were positive in 7 out of 10 population samples (Table 2), reflecting a deficit of heterozygotes. However, an apparent excess of heterozygotes was found at three sampling locations (TGN, CSP and GA). The observed negative F_{IS}, particularly for TGN and GA, is caused by the presence of many monomorphic non-informative loci, giving a greater weight to those few loci with effective heterozygote excess.

Out of 13,860 probability tests for HWE, 404 show a significance departure from *HWE*, with a nominal threshold of 0.05. However, it is noteworthy that 9,045 tests failed because loci were monomorphic within sample. Departures are not clustered by locus while, among sampling locations, GA shows the highest number of loci (151) in disequilibrium mainly for a heterozygosity excess. After correction for multiple tests, only 10 probability tests for HWE are statistically significant (3, 1 and 6 loci respectively in TGN, TP and GA populations).

3.2.2 | Inference of population structure

Two datasets were used for downstream analysis on the bases of the power analysis and neutrality test (see Supporting Information Appendix S3), but only results obtained from the original one (1,386 loci) are reported below, whenever not differently noted, as the exclusion of outliers did not change the general pattern of our results.

The AMOVA performed on all the 10 population samples showed that a high and significant percentage of the genetic variation was explained by population subdivision (F_{ST} = 0.33, $p < 0.0001$), allowing to exclude the panmixia hypothesis. Population pairwise F_{ST} values (Table 3) were always significant after correction for multiple tests suggesting a low degree of connection among all the different localities. The most divergent

TABLE 2 Genetic variability assessed at 1,386 loci within 10 *Leptopsammia pruvoti* populations and on the total dataset (N = 212). Sample acronyms are in Table 1. For each population sample, sample size (n), proportion of polymorphic loci (P_N), allelic richness (A_R), private allelic richness (pA_R), unbiased expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (F_{IS}) are reported. P_N is calculated considering the minor allele frequency (MAF) filter >1%; A_R and pA_R are calculated on a minimum sample size of 12 diploid individuals

Pop	*n*	P_N (%)	A_R	pA_R	H_E	H_O	F_{IS}
TGN	28	6.2	1.04	0.01	0.0150	0.0209	−0.4104
TR	22	35.1	1.23	0.04	0.0797	0.0741	0.0718
OT	22	40.1	1.26	0.04	0.0857	0.0820	0.0452
TP	34	41.9	1.28	0.10	0.0968	0.0922	0.0481
CSP	21	47.3	1.34	0.05	0.1205	0.1494	−0.2479
TAV	19	41.4	1.29	0.03	0.0983	0.0936	0.0492
GNS	13	33.2	1.26	0.01	0.0933	0.0870	0.0701
GNN	12	37.8	1.29	0.01	0.0976	0.0897	0.0847
PTF	26	33.6	1.24	0.04	0.0891	0.0859	0.0365
GA	15	30.1	1.22	0.02	0.0906	0.1434	−0.6200
Total	212	34.7	1.25	0.04	0.1174	0.0869	0.2605

TABLE 3 Pairwise F_{ST} matrix among *Leptopsammia pruvoti* populations based on 1,386 loci (below diagonal) and respective pairwise *p*-value (above diagonal). All F_{ST} values are associated with significant *p*-values at the 5% global level also after the Benjamini–Hochberg correction for multiple tests

	TGN	TR	OT	TP	CSP	TAV	GNS	GNN	PTF	GA
TGN	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TR	0.36	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
OT	0.39	0.14	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TP	0.54	0.41	0.41	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CSP	0.35	0.18	0.17	0.32	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TAV	0.49	0.29	0.28	0.32	0.17	–	<0.0001	<0.0001	<0.0001	<0.0001
GNS	0.58	0.32	0.30	0.35	0.19	0.18	–	<0.0001	<0.0001	<0.0001
GNN	0.56	0.30	0.28	0.33	0.17	0.16	0.04	–	<0.0001	<0.0001
PTF	0.52	0.35	0.33	0.36	0.22	0.19	0.22	0.21	–	<0.0001
GA	0.56	0.32	0.30	0.34	0.22	0.27	0.27	0.25	0.31	–

population sample was the one collected in the North Adriatic (TGN; five out of nine comparisons showing F_{ST} values >0.5) followed by the TP sample in the South Tyrrhenian. Not surprisingly, the lowest divergence was obtained when comparing the two sites in the Giannutri Island (Ligurian Sea, GNN and GNS) located at about 5 km from each other (F_{ST} = 0.04; Table 3). Accordingly, the best hierarchical AMOVA maximizing the amount of variation among groups with respect to alternative configurations was obtained considering the subdivision into 9 groups, with only the two neighbouring sampling sites of Giannutri Island (GNN and GNS) pooled together (F_{CT} = 0.29, p = 0.0245).

The DAPC, performed without reference to predefined populations, strongly supported these results (Figures 2a and 3a). In fact, almost all identified clusters strictly matched the population sampling sites (Clusters 1, 2, 5, 6, 7 and 8 in Figure 2a) with the two populations of Giannutri merged in a single cluster (Cluster 9 in Figure 2a).

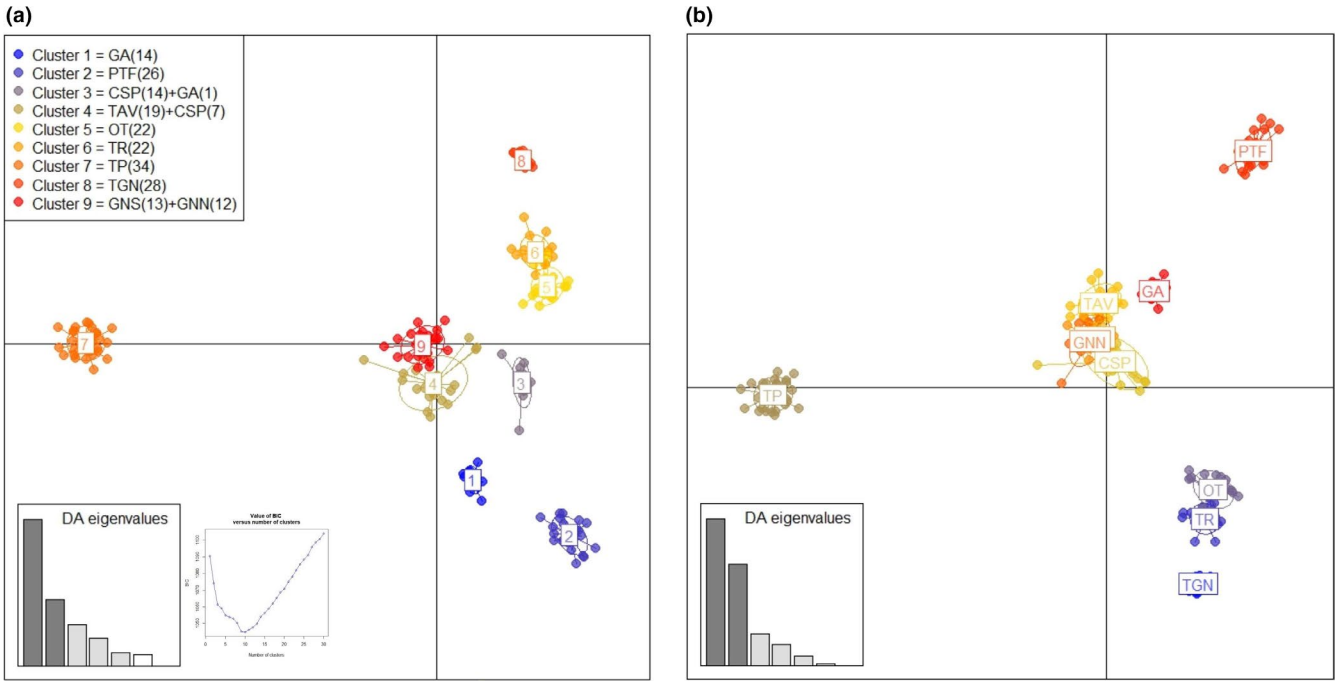

FIGURE 2 Scatterplot of Discriminant Analysis of Principal Components (DAPC) based on 1,386 loci genotyped in 212 *Leptopsammia pruvoti* individuals at 10 localities. The bar graph inset displays the variance by the discriminant eigenvalues used for plotting. The optimal number of PCs to retain was estimated (PCs = 7). (a) DAPC performed without any a priori group assignment. The estimation of the most likely number of clusters (*K*) through the *find.clusters* module is represented by values that minimize the Bayesian information criterion (BIC) displayed in inset. Nine clusters (*K* = 9) were identified. The legend reports the cluster composition, with number of individuals assigned per cluster in parenthesis. (b) DAPC performed with a priori group assignment based on sampling site. The different localities are marked with the correspondent acronym, as reported in Table 1. For both analyses, the correspondent membership probabilities of assignment for each individual are visualized as STRUCTURE-like bar plot in Figure 3

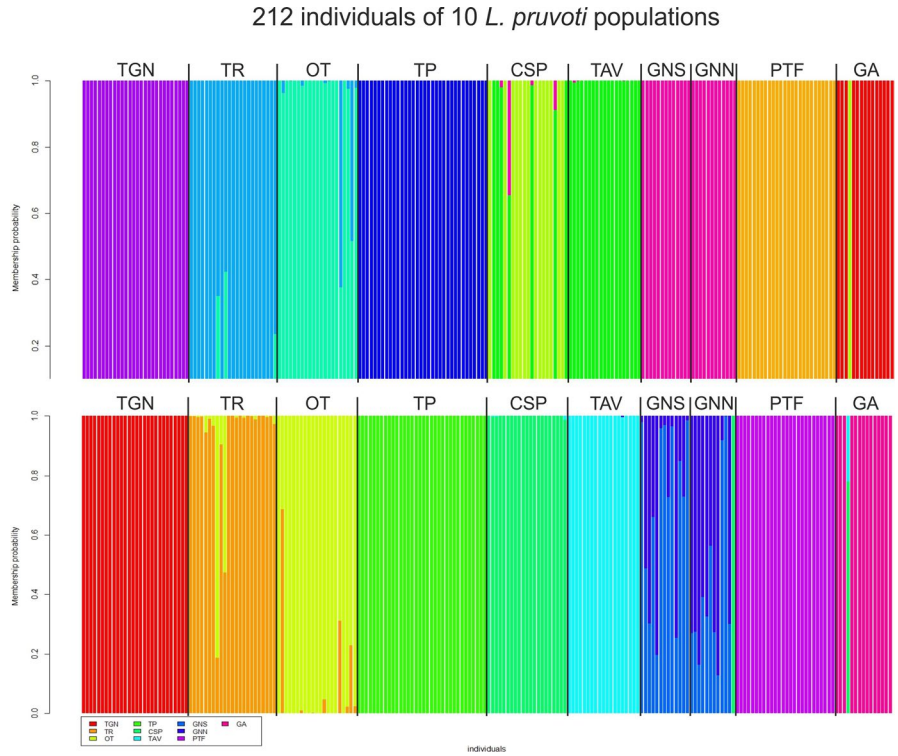

FIGURE 3 STRUCTURE-like bar plot of DAPC performed with 1,386 loci. The membership probability of cluster assignment for each individual is represented by a vertical colour bar. The bar plot (a) represents the number of clusters (K = 9) estimated by the program. Each K is identified by a group of individuals with the same colour. The number of K corresponds to clusters reported in Figure 2a in the text. The bar plot (b) represents the membership probabilities assessed considering a priori information about the sample origin (10 populations)

Interestingly, the remaining two clusters (Clusters 3 and 4 in Figures 2a and 3a) include few animals sampled in other locations. More in detail, 7 out of 21 individuals of CSP cluster within the other Sardinian site TAV (Cluster 4 in Figures 2a and 3a) while the remnant 14 individuals cluster in a separate group (Cluster 3 in Figure 3a). Another case includes one individual sampled at GA and assigned to CSP (within Cluster 3 in Figures 2a and 3a). These results suggest that, in spite of a general low level of realized dispersal, a sporadic gene flow might have occurred. The most separate of the ten clusters is entirely composed of the TP individuals (Cluster 7 in Figure 2a), collected in the only sampling site in the southern Tyrrhenian Sea.

Considering the a priori population information, the DAPC shows a clear differentiation between the eastern and western side of the Italian peninsula, better visualized in Figure 2b in which the three populations collected in the Adriatic Sea lay close to each other and separate from all the other sampling sites. Interestingly, a small level of admixture has been detected also between the Adriatic sites of TR and OT as testified by the presence in Figure 3b of individuals with mixed membership probabilities (Figure 3a).

3.2.3 | Comparison between genetic and geographical information

The correlation between genetic and geographical distances on the whole dataset resulted positive and highly significant (R = 0.707, p < 0.0001) (Figure 4). After removing the TGN population from the analysis, the correlation remains significant though less strongly supported (R = 0.473, p = 0.029), thus indicating that the high divergence of the TGN population only partially affects the result. Significance is also obtained when the correlation between genetic and geographical distances is assessed within the Tyrrhenian basin (R = 0.517 with p = 0.037). The GENELAND analyses clearly show a subdivision in four clusters corresponding to the Adriatic basin (TGN, TR and OT), the South Tyrrhenian (TP), the North-Centre Tyrrhenian (GNN, GNS, TAV and PTF) and a last cluster including the population samples of CPS and GA, collected at the most western sites (see Supporting Information Appendix S4). The presence of a clear distinction between the Adriatic and Tyrrhenian basins perfectly matches what was observed in the DAPC (Figure 2a,b) reported in the previous paragraph. Also, in the present analysis, the TP population lays separate from all the other populations.

3.2.4 | Genetic connectivity

Results of the assignment test performed with GENECLASS2 show that 184 individuals out of the 212 (86.8%) were correctly assigned to their nominal population (Table 4). About the remnant 28 misassigned individuals, the majority (26, corresponding to 12.3% of the entire dataset) were assigned to unknown sites, and only two individuals were identified as migrants. Noticeably, those

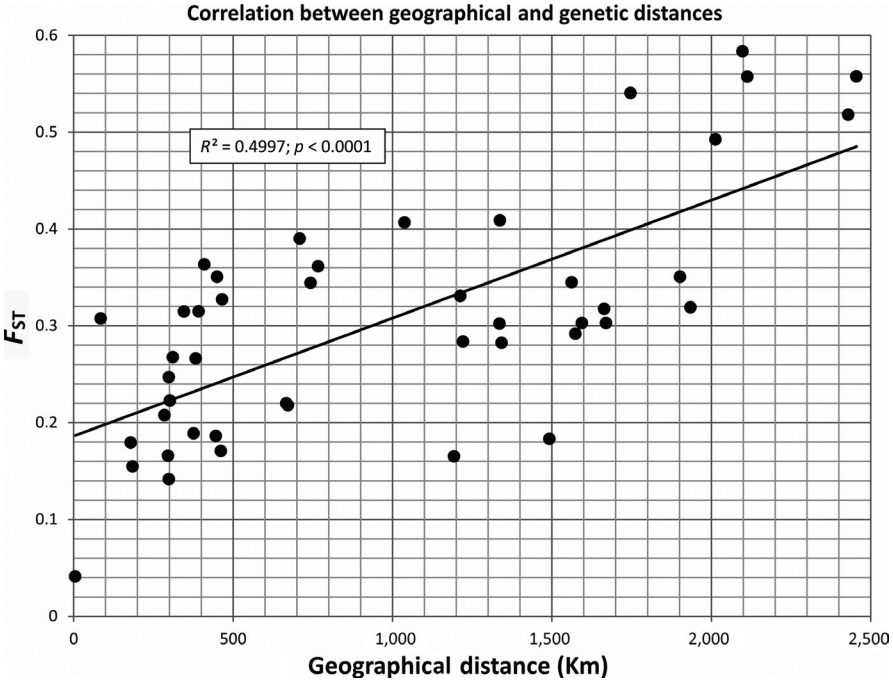

FIGURE 4 Correlation between geographical distances and genetic distances computed as F_{ST} between site pairs

Sample site	Assigned to the same pop (%)	Assigned to another population (%)	Unknown origin (%)
TGN	28 (100%)	– (0%)	– (0%)
TR	21 (95.5%)	– (0%)	1 (4.5%)
OT	19 (86.4%)	– (0%)	3 (13.6%)
TP	32 (94.1%)	– (0%)	2 (5.9%)
CSP	14 (66.7%)	– (0%)	7 (33.3%)
TAV	16 (84.2%)	– (0%)	3 (15.8%)
GNS	9 (69.2%)	2 GNN (15.8%)	2 (15.4%)
GNN	10 (83.3%)	– (0%)	2 (16.7%)
PTF	21 (80.8%)	– (0%)	5 (19.2%)
GA	14 (93.3%)	– (0%)	1 (6.7%)
Total	184 (86.8%)	2 (0.9%)	26 (12.3%)

TABLE 4 Assignment tests of the individuals of *Leptopsammia pruvoti* based on the dataset including 422 loci shared by all individuals and implemented through the Bayesian exclusion method of Rannala and Mountain (1997). The table shows the number and percentage of individuals that get assigned to their nominal population (second column) and to another population (third column). Individuals that could not be assigned to any sampled populations are in the column "Unknown origin."

two individuals were collected at GNS and assigned to the closest site GNN, only 5 km away, supporting the hypothesis of possible dispersal between neighbour areas despite high percentages of self-recruitment typical of this species, as also testified by the remarkable genetic structure observed (Figures 2a,b and 3a,b). The TGN population resulted to be the most isolated with all individuals correctly reassigned to the location of origin. This could indicate a high incidence of self-recruitment in this population at the edge of the species distribution area. Interestingly, the CSP population was split into two groups, the first with 14 individuals correctly assigned to their sampling origin and the remnant seven individuals assigned to an unknown site. The same seven animals clustered with TAV using DAPC (Figures 2a and 3a), suggesting direct immigration. However, the comparison between TAV and the seven individuals from CSP resulted statistically significant using F_{ST} (F_{ST} = 0.14, p < 0.0001, Supporting Information Appendix S5). Overall, our results strongly suggest a mosaic composition of the CSP population sample.

4 | DISCUSSION

Overall, our 2b-RAD population genomic analysis provides clear evidence for strong genetic differentiation among Mediterranean population samples of the scleractinian coral *L. pruvoti*. Using over one thousand SNPs and samples from ten populations collected along the Italian coastline at distances ranging from tens to thousands of kilometres, we detected significant differences among all the sites, a frequent heterozygote deficiency, a high incidence of self-recruitment with occasional dispersal between neighbour areas, and a

large-scale subdivision between the western (Ligurian–Tyrrhenian Sea) and the eastern side (Adriatic Sea) of the Italian peninsula.

These results apparently contrast with an early report on this species based on allozymes that failed to detect a significant correlation between genetic differentiation and geographical distance and evidenced a stronger genetic differentiation at the local scale than among distant populations (Goffredo et al., 2009).

However, our results confirm those obtained on other brooding scleractinian coral species with a similar life cycle (Casado-Amezùa, Goffredo, Templado, & Machordom, 2012; Maier, Tollrian, Rinkevich, & Nurnberger, 2005; Ridgway, Riginos, Davis, & Hoegh-Guldberg, 2008) suggesting that an extreme genetic differentiation even at local scale might be a general feature of these species. These results should be taken into account in future conservation strategies and require a careful spatial prioritization reflecting genetic diversity and potential for connections.

4.1 | Strong genetic differentiation in *L. pruvoti*

A very clear and congruent pattern of strong population structure emerges from our data. All the pairwise F_{ST} values were significantly different, ranging from above 0.5 for some comparisons involving the North Adriatic sample from TGN to a minimum of 0.04 for the comparison between the two samples collected at 5 km distance, on the same island of Giannutri (GNS and GNN), in the Tyrrhenian Sea (Figure 1 and Table 3).

This high level of genetic differentiation is fully consistent with the results of DAPC (Figures 2 and 3), and with the high level of self-assignment detected by assignment tests (Table 4), which showed that from 80.8% up to 100% of individuals were consistent with a local origin. Excluding the very close samples from Giannutri (GNN and GNS) that are the less differentiated samples, the only exception to this pattern was for the population sample collected at CSP, which resulted to have only 66.7% of self-recruitment and showed a mosaic composition comprising two distinct clusters of individuals with DAPC (Clusters 4 and 6 in Figures 2 and 3). Similarly, significant differences have been reported at the local scale by Goffredo et al. (2009). In our case, this mosaic composition likely results from immigration, with DAPC indicating TAV as source population for the seven individuals of Cluster 6, whereas assignment test suggested that they might originate from an unsampled source population (Table 4). This result awaits confirmation as the fine-scale individual mapping of CSP samples was not performed in this study.

A high level of self-assignment, in a context where strong genetic differences occur at small geographical distances, is a strong indication of self-recruitment and genetic drift as dominant drivers explaining the genetic differentiation between *L. pruvoti* populations. Indeed, the predominance of local reproduction, when considered together with the low genetic variability (Table 2), suggests the existence of small populations that can experience strong genetic drift. From this point of view, a marked departure from HWE and considerable deficit of heterozygotes have been recorded in *L. pruvoti* using allozymes (Goffredo et al., 2009) and partially confirmed in our study where most of the samples showed positive inbreeding coefficients, though our SNP loci were generally in HWE. The observed negative F_{IS} at three samples (Table 2) may be an artefact, due to the presence of monomorphic non-informative loci, which inflate the estimate due to few loci with heterozygote excess, though we cannot exclude some level of clonality (see Results2.4). The hypothesis of a localized recruitment with consequent biparental inbreeding (Goffredo et al., 2009) is especially interesting when considering the TGN population, which besides being the most differentiated sample also presents the lowest level of genetic variation. The observed northward decrease of both allele richness and heterozygosity raises the intriguing hypothesis that the genetic variability of the Adriatic samples still keeps traces of the colonization processes that occurred in the last 10–5 ka with an expected lower diversity towards the edge of the distributional range of the species. Moreover, the fragmented distribution of suitable habitats in the North Adriatic region is expected to further reduce the connectivity among local populations. This scenario requires further testing, as the observed pattern could also be due to an occasional long-distance dispersal to the single northern Adriatic site. A fine-scale analysis of *L. pruvoti*, based on more population samples, would allow comparing the distribution of genetic variability with the palaeo-geographical patterns of post-glacial flooding of the Adriatic region to understand whether this species, for its biological features, potentially acts as a reliable witness of past colonization processes.

A higher genetic variability and a remarkable number of private alleles found in the Ligurian–Tyrrhenian Sea (up to 8% in the TP sample) suggest an older origin and a higher stability of those populations, pointing to a long history of isolation that allowed specific mutations to appear and accumulate locally.

In spite of the low dispersal capability of this species, a certain degree of gene flow seems to take place, according to the assignment tests, which directly detected two first-generation migrants between the two closest population samples of GNN and GNS, and from the existence of few individuals of mixed origin in the Adriatic samples (Figure 3). Interestingly, and similarly to what was observed in other species (Casado-Amezùa et al., 2012; Postaire, Gélin, Bruggemann, & Magalon, 2017), this gene flow must be strong enough to maintain a significant correlation between geographical and genetic distances but limited enough to prevent genetic homogenization.

The observed differentiation among Ligurian–Tyrrhenian Sea and Adriatic Sea as well as the clear distinction of the TP population, evident also in DAPC and GENELAND analysis (Figures 2 and 3), apparently reflects a general pattern of zoogeographical differentiation already observed in other species. This result awaits confirmation, as our sampling did not include samples either from the Ionian Sea, which might represent a transition zone between the two basins (Costantini et al., 2018; Dailianis, Tsigenopoulos, Dounas, & Voultsiadou, 2011; Villamor, Costantini, & Abbiati, 2018), or from the Tunisian. A similar pattern of differentiation among Ligurian–Tyrrhenian Sea, Adriatic Sea and Sicily channel samples has been reported in several species, including invertebrates and fish, though

with a much lower level of differentiation (Congiu, Rossi, & Colombo, 2002; Maltagliati, 1999; Marino, Pujolar, & Zane, 2011).

Occasional long-range dispersal events may occur in marine invertebrates (Hellberg, 2009). This was also observed, in our species, within the mosaic composition of the CSP sample, in which seven individuals clearly originated from another population that, according to DAPC, has a genetic composition similar to TAV one (Figures 1–3).

Leptopsammia pruvoti broods larvae and releases planulae, which are small, completely differentiated and ready to settle, but with a ciliary movement, neutral buoyancy and pelagic dispersal that, in aquaria, can last up to 20 days (Goffredo et al., 2005). The low level of immigration here detected at the local scale suggests that the efficiency of movement to other areas remains small, but not negligible. Accordingly, genetic results show that most larvae set directly in the natal population, probably as soon as a suitable substrate is identified. Only a small proportion moves to close areas, and very rarely to distant populations. It is known, in fact, that most brooders, even with very short pelagic larval duration, show larvae capable of settling many weeks after the planktonic phase in case of suboptimal substrates, thus suggesting the possibility of sporadic migration event at larger scale (Ayre & Hughes, 2000 and references therein) under favourable oceanographic conditions, as suggested for the Capo Spartivento population. In conclusion, most data here reported are congruent with the hypothesis of a restricted gene flow expected in brooder corals (Carlon, 1999).

4.2 | Comparisons with other species

In marine organisms, an extended larval phase can play an important role in determining wide distributions (Lester, Ruttenberg, Gaines, & Kinlan, 2007; Luiz et al., 2013), and despite the vagaries associated with the correlation of genetic differentiation and pelagic larval duration (PLD; Selkoe & Toonen, 2011; Weersing & Toonen, 2009), it is often associated with a low genetic differentiation. A short larval phase, on the contrary, could prevent long-range dispersal leading to high cryptic diversity (Postaire et al., 2017).

For instance, when considering fish with a Mediterranean–Atlantic distribution, a weak differentiation is often found when comparing populations sampled at thousands of kilometres of distance (i.e., Milano et al., 2014; Souche et al., 2015), and extreme differences are found at most across major biogeographic/oceanographic transitions (Patarnello, Volckaert, & Castilho, 2007 and references therein). Indeed, for marine fish, F_{ST} values rarely exceed 0.1–0.15 within the Mediterranean Sea, even when considering species with a limited PLD, fragmented habitat and endemism level (Astolfi et al., 2005; Carreras et al., 2017; Carreras-Carbonell, Macpherson, & Pascual, 2006; Congiu et al., 2002), and only raise to high values when considering sharks (Ashe et al., 2015), reptiles (Sethuraman et al., 2013) and some marine mammals (Fruet et al., 2014). Similar pictures of low differentiation are common in several benthic invertebrates, such as sponges (*Scopalina lophyropoda*, Blanquer & Uriz, 2010), sea urchins (*Paracentrotus lividus*, Maltagliati, Di Giuseppe, Barbieri, Castelli, & Dini, 2010, Penant, Aurelle, Feral, & Chenuil, 2013), sea cucumbers (*Holothuria mammata*, Borrero-Pérez, Gonzàlez-Wanguemert, Marcos, & Pérez-Ruzafa, 2011), bivalves (*Pinna nobilis*, Sanna et al., 2013) and crustaceans (*Liocarcinus depurator*, Pascual et al., 2016; *Carcinus aestuarii*, Schiavina, Marino, Zane, & Melìa, 2014; *Pachygrapsus marmoratus*, Fratini et al., 2016).

With reference to the specific area of interest, some recent studies already demonstrated a signal of differentiation, even though generally weak, in different key marine species with different distribution capacity, suggesting in some cases an agreement with Lagrangian simulations and/or network reconstruction. Paterno et al. (2017) depicted, using SNPs, a picture of low differentiation, even if significant, between the western Mediterranean Sea and Adriatic Sea for populations of *Paracentrotus lividus*, consistent with its potential larval dispersal obtained via Lagrangian simulations and with results obtained by several other studies (Borsa, Blanquer, & Berrebi, 1997). On the contrary, populations of the Mediterranean shore crab *Carcinus aestuarii*, besides showing the higher differentiation between Tyrrhenian and Adriatic basins with F_{ST} ranging between 0.027 and 0.039, have shown also a weak but significant structure into three clusters within the Adriatic Sea (northern, central and southern) through microsatellite analysis (Schiavina et al., 2014). As well, Carreras et al. (2017) observed a barrier to gene flow as responsible for the differentiation between the central (Tremiti) and the southern Adriatic Sea (Otranto) with an F_{ST} of 0.0138 using SNPs in the East Atlantic peacock wrasse (*Symphodus tinca*), consistent with what reported for mackerels (Papetti et al., 2013), but still far from what was observed here for *L. pruvoti*.

The picture here presented, however, might be less unusual when considering other habitat-forming species or species strictly associated with them. More specifically, significant genetic differentiation at all spatial scales has been found in most sessile marine invertebrates. Among these, taking into account that different markers have been used and that comparisons are only indicative, we cite the populations of the clonal soft coral *Alcyonium rudyi* in north-western coast of North America (F_{ST} = 0.23–0.46; McFadden, 1997), populations of the viviparous corals *Seriatopora hystrix* in the Australian Great Barrier Reef (F_{ST} = 0.43; Ayre & Dufty, 1994), and the solitary cup coral *Balanophyllia (Balanophyllia) elegans* in the Pacific Coast of North America (F_{ST} = 0.28; Hellberg, 1994). Notably, most of them are brooding corals whose larvae typically settle within few days after planulation. A similar level of differentiation and an IBD pattern were detected in a brooding species, the hydrozoan *Lytocarpia brevirostris*, in the Western Indian Ocean and New Caledonia (Postaire et al., 2017). Similarly, the coral-excavating sponge, *Cliona delitrix*, which broods and releases short-lived larvae, exhibits strong genetic differentiation in the Caribbean Sea and in the Atlantic (Chaves-Fonnegra, Feldheim, Secord, & Lopez, 2015). Looking at studies focusing on the Mediterranean basin, a similar pattern of differentiation, high level of self-recruitment and low connectivity have been found in brooder coral such as the red gorgonian *Paramuricea clavata* (F_{ST} = 0.116; Mokhtar-Jamai et al., 2011), the orange coral *Astroides calycularis* (F_{ST} = 0.236; Casado-Amezùa et al.,

2012) and the red coral *Corallium rubrum* showing pairwise F_{ST} ranging from 0.01 to the higher value of 0.30 at a small spatial scale in the Ligurian Sea (Costantini, Fauvelot, & Abbiati, 2007; Costantini et al., 2011) and in Sardinian waters (Cannas et al., 2015,2016). Our study, as already observed in other scleractinian corals, seems to confirm the correlation between a strong population differentiation among locations and a short larval duration. These evidences are probably even more pronounced in brooder organisms.

4.3 | Implications for conservation

Estimating population structure, pattern of connectivity and self-recruitment in marine populations is essential to study, design and implement MPAs and their networks. Networks of MPAs, in particular, should be implemented in the ecological space within Cells of Ecosystem Functioning (Boero et al., 2016), units of management and conservation, where biodiversity patterns and ecosystem processes are spatially linked by high connectivity.

Several studies have recently accepted that the management of MPA networks should consider the ecosystem as a whole, by including species with different PLD. In species with long PLD, in fact, larvae move for days and currents and/or other oceanographic features significantly affect their dispersion. For these species, the resulting pattern of geographical distribution of genetic diversity often reflects the presence of oceanographic discontinuities. On the contrary, in species with shorter PLD, the potential dispersal capability can be very low and a high genetic differentiation is expected also at small geographical scale, independently of the absence of evident oceanographic barriers. Other aspects that can further shape the structure of the different species are the distribution and the fragmentation of suitable habitats for the different species. For these reasons, species with different life history and ecology should be included into an integrated multispecies approach to implement functional and resilient conservation networks (Melià et al., 2016; Pascual et al., 2017).

A special attention should be devoted to species for which, besides a low PLD, other life history traits such as the sessile adult stage and self-recruitment seem to strengthen the expected strong population differentiation and low gene flow regardless of existing marine fronts (Pascual et al., 2017). Clearly, *L. pruvoti* is a species with very low dispersal potential. Moreover, its widespread distribution in the Mediterranean Sea and a good ecological tolerance in terms of temperature range (Caroselli et al., 2012) make it a priority candidate to be included in a panel of selected umbrella species that should be considered in performing a multispecies connectivity assessment in the Mediterranean Sea, as suggested by Pascual et al. (2017). The expected high degree of differentiation has been here confirmed in 10 populations of *L. pruvoti*, emphasizing the need of management activities tailored at local scale on the specific features of this species. In hindsight, the same approach should have been applied also at a smaller geographical distance to assess the real degree of connectivity. However, although more species should be genetically assessed to better describe the existing variability and the pattern of connectivity among bioconstruction communities, the present paper allows proposing some general criteria to be used for the establishment of the MPAs when bioconstructors are considered. First, investigations about the connectivity among populations of bioconstruction species should be designed by including samples from very close geographical areas, to have a reliable estimate of the real dispersal ability of the different species. Second, dealing with bioconstructors, strong emphasis should be given to the limited connectivity. The chances for migrants to successfully reach suitable and well-preserved substrates for settlement should be maximized by increasing the number of MPAs, with consequent reduction of the distances among *reservoirs* that should not exceed the spatial scale of tens of kilometres. To this regard, a good knowledge of coastal oceanography might be critical as very closed areas might be actually isolated by specific current dynamics. Third, areas in which bioconstructor species are already disappearing, mainly due to human impact, must be prioritized in terms of restoration as their local extinction would be hard to recover, considering the very low dispersal capabilities estimated.

4.4 | Conclusions

The highly supported results confirmed the usefulness of the 2b-RAD approach as an effective tool to genotype a high number of SNP markers across the entire genome in non-model organisms, with no need for a reference genome. Thus, this method can be especially useful to study population genomics in marine invertebrates for which no, or little, a priori genetic information is available.

We proposed the first overview of an important exponent of the Mediterranean coralligenous formations, which is actually considered as the second most important marine habitat type to be characterized in view of future plans for the management of MPAs.

The strong pattern of genetic differentiation among all populations of *L. pruvoti* is probably the result of its life history traits. Although these evidences should be confirmed in other species before generalizing this pattern, the work provides useful clues for the management of the Mediterranean basin and criteria for the identification of new areas needing protection.

Our results indicate that extensive dispersal and massive export might not be the rule: we suggest that fine-scale resolution data (few kilometres) should drive conservation planning when the target of conservation, restoration and management is bioconstruction species with low PLD in order to optimize their protection by selecting the appropriate distance and size of MPAs and to eventually direct suitable restoration measures.

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DATA ACCESSIBILITY

The dataset supporting the conclusions of this article is available in genepop format as additional file in DRYAD at https://doi.org/10.5061/dryad.9hb966d. Short read data are deposited in SRA with accessions SRR8648865–SRR8649076.

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BIOSKETCH

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Author contributions: EB, IAMM, LC and LZ conceived and designed the experiments and wrote the manuscript. EB performed the experiments. EB, IAMM, CP, LC and LZ analysed the data and contributed reagents/materials/analysis tools. MB, FB, GB, LBC, RC, AC, CC, GC, FC, SF, GI, FM and MP contributed to sampling. All authors revised the article critically and approved the final version to be published.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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